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INTRODUCTION

Akt is a serine/threonine protein kinase functioning downstream of PI3K in response to mitogen or growth factor stimulation [1]. Three isoforms of Akt are present in mammalian cells, Akt1/PKB α and Akt2/PKB β , and Akt3/PKB γ [2-4]. Threonine 308 and Serine 473 are phosphorylated by PDK1 and PDK2, respectively [5,6]. The tumor suppressor gene PTEN or MMAC1 acts as a lipid phosphatase for phosphatidyl inositol at 3' [7,8] and SHIP is another phosphatase for phosphatidylinositol at the 5' position [8-10]. Both of these proteins negatively regulate the activity of Akt/PKB by depleting the pool of phosphatidyl inositol 3,4,5-trisphosphates. Except its roles in cell metabolism [12, 13], inappropriate activation of AKT/PKB has been associated with the development of cancer [5, 14,15] and the resistance of cancer cells to chemotherapy [16], which include leukemia [17], ovarian cancer [18], breast cancer [19], prostate cancer [20, 21], glioma [22], pancreatic cancer [23], renal cancer [24], hepatoma [26], and lung cancer [26]. Recent studies have also shown that activation of AKT promotes the metastasis of cancer cells [27-29]. It looks like that the hormone-dependency results in the activation of AKT, at least in breast and prostate cancer cells. It has been shown that AKT3 is 20-60 fold higher in estrogen receptor-deficient breast cancer cells and in the androgen-insensitive prostate cells than in the cells that are estrogen- or androgen-responsive [30]. It was also shown that the mRNA level of AKT3 is elevated in hormone insensitive breast cancer cell lines and prostate cancer cell lines. Furthermore, the activity of AKT3 is 40-100 fold elevated in PC-3 cells that do not have the tumor suppressor PTEN in comparison to DU-145 cells that do have PTEN [31-34]. On the other hand, androgen receptor expression is regulated by PI3-K/AKT in normal and tumor epithelial cells [35]. p53 plays a major role in regulating the response of mammalian cells to stresses and damage, in part through the transcriptional activation of genes involved in cell cycle control, DNA repair, senescence, angiogenesis and apoptosis [36]. Recent observations show that PI3K-Akt signaling promotes the phosphorylation and movement of the Mdm2 oncoprotein into the nucleus, where it downregulates the p53 tumor-suppressor protein [37-41]. Akt has been shown to affect the apoptotic processes by multiple mechanisms involving inhibiting pro-apoptosis protein Bax conformational change [42], phosphorylation of several other components of the apoptotic machinery, including BAD [43] and caspases 9 [44] at a postmitochondrial level [45]. AKT also cooperates with anti-apoptosis protein Bcl-X_L to promote cell survival or cancer development [46, 47]. In addition, AKT modulates apoptosis indirectly by influencing the activities of several families of transcription factors, including fork head transcription factor, NF- κ B, and cyclic AMP-responsive element binding protein [5]. One other reason for AKT activation promoted cancer development is that AKT activation increases mammalian cell size by stimulating protein synthesis as well as by inhibiting protein degradation [48], through activation of downstream protein synthesis signal transduction pathway via TSC1-TSC2 (tuberous sclerosis complex) [49, 50]. These studies have established targeting AKT as an attractive strategy for cancer therapy [51]. To date, there is no report of selective inhibitors targeting AKT. It is of note that in recent years, highly selective and potent kinase inhibitors have been successfully designed including kinase inhibitors targeted at the EGFR family kinase, a number of these kinase inhibitors have advanced into clinical trials for the treatment of cancers [52,

53].

The objectives of this project are 1. Structure-based discovery of potential AKT kinase inhibitors. 2. Investigation of the potency and selectivity of AKT kinase selective inhibitors. 3. *in vivo* antitumor activity and toxicity

Body of Report

Accomplishments on Objective 1: Structure-based discovery of potential AKT kinase inhibitors

Beyond the scope of this original objective, to further increase the chance to screen for selective AKT inhibitors, we also established and successfully utilized a bioinformatics-based approach:

1.1. Characterization of the Expression Status of AKT and PTEN in NCI 60 Cancer Cell Lines

Since Akt plays such a important role in the development of cancer, the development of resistance to chemotherapeutic regents of cancer cells, and metastasis of cancer cells, we characterized the expression status of Akt, phospho-specific AKT, and PTEN in NCI 60-cancer cell lines with Western blot analysis using Akt (recognizes all three isoforms of Akt), phospho-Ser473-AKT, and PTEN antibodies, respectively. As exhibited in figure 1, there is no difference among NCI-60 cancer cells regard to their AKT expression (data not shown), but the expression of phospho-Ser473-AKT and PTEN are quite different in these 60 cell lines. Among them, SNB19 and SF-539 glioma cancer cells express the highest level of phospho-Ser473-AKT, whereas they do not express PTEN or express undetectable level of PTEN; HS578T breast cancer cells expresses high level of phospho-Ser473-AKT, but it expresses a relatively high level of PTEN; MCF-7 breast cancer cells do not express phospho-Ser473-AKT, but it expresses the highest level of PTEN. MDA-MB-453 breast cancer cell line, one of the model cell lines used in this work also expresses high level of AKT and PTEN (data not shown). These results suggest that the expression of phospho-Ser473-AKT, which also stands for phospho-Thr308-AKT, is unique in each of the cancer cell lines and can be used as a target for drug development.

1.2. Bioinformation-based Correlation Analysis

Algorithms Description

We have developed three algorithms in JAVA to measure the distribution agreement between the expression status of molecular target and the antiproliferation patterns of NCI small molecule organic compounds against NCI 60 human cancer cell lines. It is assumed that such a higher correlation coefficient indicates the small molecule candidate might be a possible inhibitor of that molecular target.

1.2.1. Pearson's Correlation Analysis Algorithm

As a linear correlation method, Pearson's correlation algorithm has been used widely [54, 55]. To estimate the association between two variables (x_i, y_i) that are ordinal or continuous, the linear correlation coefficient r (also called Pearson's r) is given by the formula:

$$r = \frac{\sum_{i=1}^N (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^N (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^N (y_i - \bar{y})^2}} \quad (1)$$

where, N is the pairs of measurements (x_i, y_i) . \bar{x} and \bar{y} are the mean of x_i 's and y_i 's, respectively.

Pearson's correlation method has been applied in NCI's COMPARE program [56-62]. However, the value of r can be completely meaningless if the joint probability distribution of the given variables x and y are too different from a binormal distribution. Therefore, we have also developed two additional algorithms, based upon the concepts of nonparametric or rank correlation.

1.2.2. Spearman's Correlation Analysis Algorithm

Let R_i and S_i be the rank of x_i and y_i among the other x_i 's and y_i 's, respectively. When there are ties, let f_k be the number of ties in the k^{th} group of ties among the R_i 's, g_m be the number of ties in the m^{th} group of ties among the S_i 's. Then the Spearman's rank-order correlation coefficient r_s is defined as [54, 55]:

$$r_s = \frac{1 - \frac{6}{N^3 - N} [D + \frac{1}{12} \sum_k (f_k^3 - f_k) + \frac{1}{12} \sum_m (g_m^3 - g_m)]}{\sqrt{1 - \frac{\sum_k (f_k^3 - f_k)}{N^3 - N}} \sqrt{1 - \frac{\sum_m (g_m^3 - g_m)}{N^3 - N}}} \quad (2)$$

where, D is the sum squared difference of ranks, defined as

$$D = \sum_{i=1}^N (R_i - S_i)^2 \quad (3)$$

1.2.3. Kendall's Correlation Analysis Algorithm

Kendall's correlation method is even more nonparametric than Spearman's [54, 55]. Instead of using the numerical difference of ranks, it only employs the relative ordering of ranks: higher in rank, lower in rank, or the same in rank. For the N data points (x_i, y_i) , we consider all $C_N^2 = \frac{1}{2} N(N-1)$ pairs of data points, where a data point cannot be paired with itself, and where the points in either order count as one pair. We call a pair "concordant" if the relative ordering of the ranks of the two x 's (e.g. x_i and x_j) is the same as the relative ordering of the ranks of the two y 's (e.g. y_i and y_j). We call a pair "discordant" if the relative ordering of the ranks of the two x 's is the opposite from that of the two y 's. If there is a tie in either the ranks of the two x 's or the ranks of the two y 's, we will call the pair an "*extra_y*" pair when the tie is in the x 's, or an "*extra_x*" pair when the tie is in the y 's. Kendall's τ is the following simple combination of these various counts:

$$\tau = \frac{\sum \text{concordant} - \sum \text{discordant}}{\sqrt{\sum \text{concordant} + \sum \text{discordant} + \sum \text{extra}_y} \sqrt{\sum \text{concordant} + \sum \text{discordant} + \sum \text{extra}_x}} \quad (4)$$

Since Kendall's τ uses a "weaker" property of data, it is more robust than Spearman's r_s to predict the distribution agreement between two variables. On the other hand, it throws away too much information that is available to r_s , it is less powerful than r_s . In some case, τ fails to find an actual correlation that r_s does find. On balance, Spearman's r_s is preferred as being the more straightforward nonparametric test, but both statistics are in general use, as well as the parametric method, Pearson's correlation algorithm.

Correlation Analysis

The correlation between phospho-Ser473 AKT expression profile and the antiproliferation patterns of NCI small molecule organic compounds against NCI 60 human cancer cell lines has been individually analyzed using the three algorithms as described above. In our case, x is the experimental value of phospho-Ser473 AKT or PTEN expression level, and y is the $-\log GI_{50}$ value of the NCI compound. We have downloaded the $-\log GI_{50}$ data of 37,000 small molecules from DTP website [57], and put them into a database for high throughput screening. The mean bar chart of the phospho-Ser473 AKT and PTEN expression status in NCI cancer cell lines is illustrated in Figure 1. According to our correlation analysis, 17 compounds have been predicted as phospho-Ser473 AKT inhibitor candidates by all three algorithms. Among them, NSC 632855 (API-59) shows a nice correlation with all three algorithms, which correlation coefficient is shown in table 1. The chemical structure and anti-proliferative activity data against NCI 60 human cancer cells are illustrated in figure 2. Our following studies show API-59 does inhibit phospho-Ser473 AKT. On the other hand, with a reverse correlation evaluation between PTEN expression level and anti-proliferative data of NCI compounds, API-59 gives an insignificant correlation coefficient in all three algorithms (data not shown), this suggests API-59 is an AKT inhibitor, but may not be a potent PTEN agonist.

Table 1. Correlation coefficient of API-59 with three algorithms

Algorithms	Correlation Coefficient
Pearson's	0.4215
Spearman's	0.4083
Kendall's	0.3199

Accomplishments on Objective 2: Investigation of the potency and selectivity of AKT kinase selective inhibitors

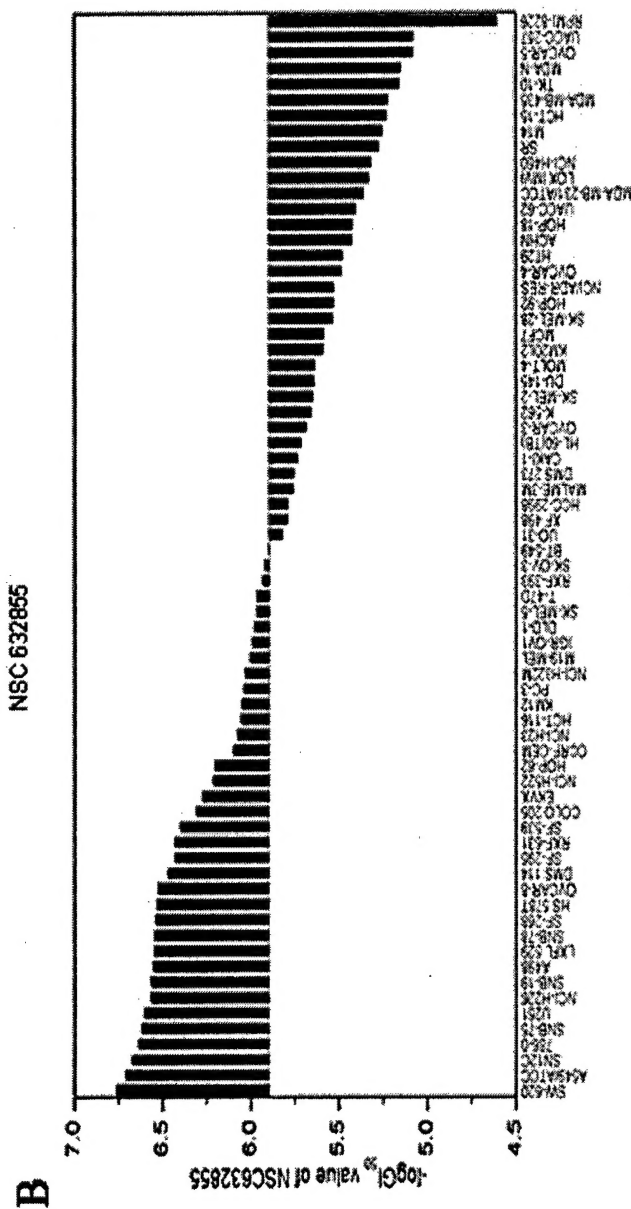
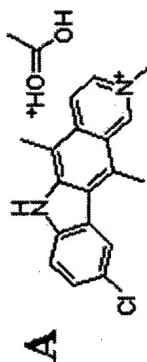


Fig. 2 A. Chemical structure of lead compound API-59 (NSC 632855 (*2-chloro-2, 5,11-trimethyl-6H-2-pyrido[4,3-*b*]carbazole acetate*)) predicted by correlation analysis methods. **B.** Mean Bar Chart of the Activity Profile of NSC 632855 against NCI 60 Human Cancer Cell Lines. $-\log_{10}$ GI50 Value through the Courtesy of Developmental Therapeutics Program, National Cancer Institute. Standard dev. = 0.505, Mean = 5.896 (1.3 uM), Max = 6.755 (175.8 nM), Min = 4.602 (25.0 uM).

In addition to Akt kinase activity assay, we modified and successfully utilized an ELISA and fluorescence polarization-based kinase assays to detect and confirm the activities of potential Akt inhibitors in inhibition of the Akt kinase activity, then using our routine cell-based assays to evaluate the potency and selectivity of Akt inhibitors in inhibition and induction of apoptosis in cancer and normal cell lines.

2.1. Identification of Akt inhibitors with AKT kinase assay, ELISA, and Fluorescence Polarization-based IMAPTM kinase assay

We utilized a sensitive and quantitative *in vitro* AKT kinase assay (New England Biolab) to identify cell lines with highest level of AKT kinase activity. The principle behind this assay is to use an antibody against AKT to selectively immunoprecipitate AKT from cell lysates. The resulting immunoprecipitated enzyme is then incubated with GSK-3 fusion protein in the presence of ATP and kinase buffer. In this reaction mixture, GSK-3 is phosphorylated and subsequently detected by western blot with a Phospho-GSK-3 (Ser21/9) antibody. Selective analysis of GSK-3 phosphorylation at Ser21/9 gives rise to an improved specificity with high sensitivity and near zero background. This assay allowed us to screen the potential inhibitors under either cell-free condition (adding the inhibitors directly in the kinase reaction mixture) or in the intact cells (treating cells in culture medium with inhibitors first).

Using this method, we immunoprecipitated AKT (monoclonal antibody 1G1,

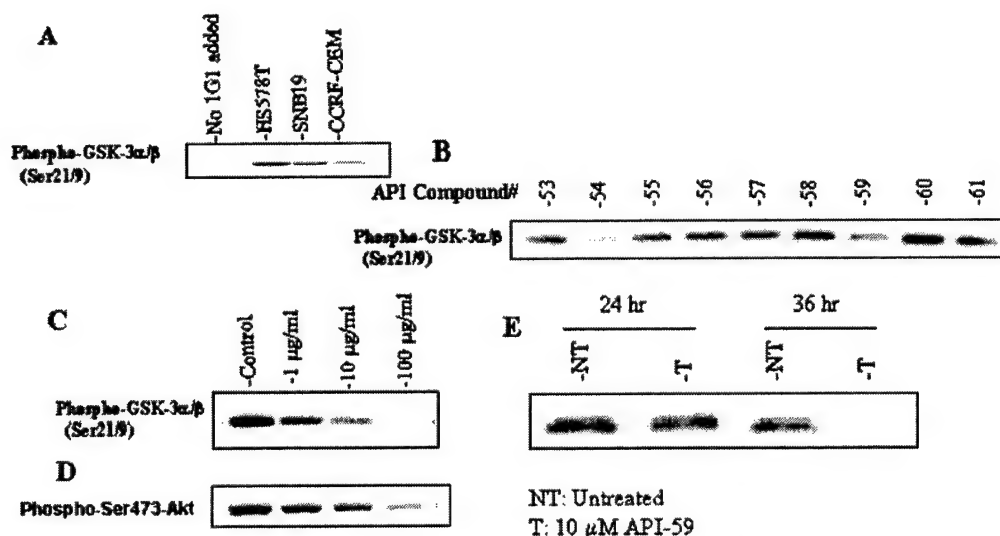


Fig. 3 A: HS578T shows the highest AKT activity. 100 μg lysates of HS578T, SNB19, and CCRF-CEM cells were subjected to AKT kinase assay. **B:** AKT inhibitor (API) screening with AKT kinase assay 100 μg lysates of HS578T cells were immunoprecipitated with 1G1 AKT antibody, the immunoprecipitated AKT was used to conduct AKT kinase assay in the presence of 10 μg/ml of different AKT inhibitor candidates. **C:** Dose-Dependent AKT inhibition by API-59. 2×10^5 HS578T cells were treated with desired concentrations of API-59 for 30 min, cell lysates were harvested and subjected to AKT kinase assay. **D:** API-59 inhibits phospho-Ser473-Akt. Same lysates were subjected to Western blot analysis with phospho-Ser473-Akt antibody. **E:** Time-course of the inhibitory effect of API-59. 2×10^5 HS578T breast cancer cells were treated with 10 μM API-59 for various times, cell lysates were subjected to AKT kinase assay.

recognizes AKT1, AKT2, and AKT3) from breast cell line Hs578T which has highest level of activated AKT kinase (Fig. 3A) to screen a total of 69 candidate small molecule

inhibitors from top-200 compounds with good correlation in three algorithms, as shown in **figure 3B**. We found 5 compounds displayed a specific kinase inhibition for AKT with an IC_{50} from 1 to 10 $\mu\text{g/ml}$. In order to test the cell permeability of those compounds, we then carried out the cell based AKT kinase inhibition assay by treating HS578T cells in culture with those 5 lead compounds. One of which **API-59**, has cellular IC_{50} value at about 10 $\mu\text{g/ml}$. A dose and time-dependent inhibition of **API-59** is shown in **Fig. 3C and 3E**. Inhibition of AKT kinase activity also led to reduction of phosphorylation of AKT as shown in **Fig. 3C**, when the same cell lysates was investigated with phospho-Ser473 AKT antibody by western blot analysis.

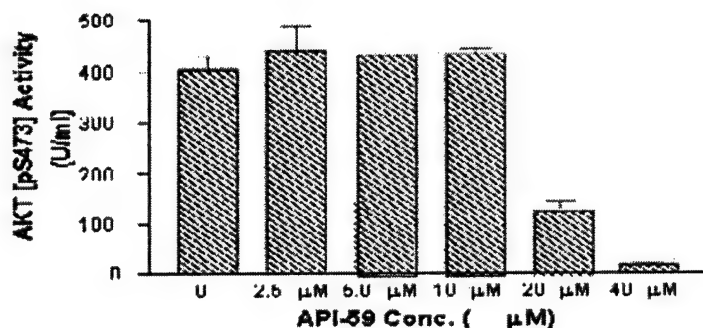


Fig. 4 1×10^6 MDA-MB-453 cells were treated with API-59 for 24 hrs, cells lysates were subjected to an ELISA-based AKT [pS473] activity assay.

To verify API-59 does inhibit phospho-Ser473 AKT, we conducted an ELISA based assay to see whether API-59 inhibit the activity of phospho-Ser473 AKT in MDA-MB-453 cells. As demonstrated in **figure 4**, treated with various concentration of API-59 for 24 hrs, the activity of phospho-Ser473 AKT went down at 20 μM of API-59 treatment, 40 μM of API-59 treatment was even able to block all the activity of phospho-Ser473 AKT.

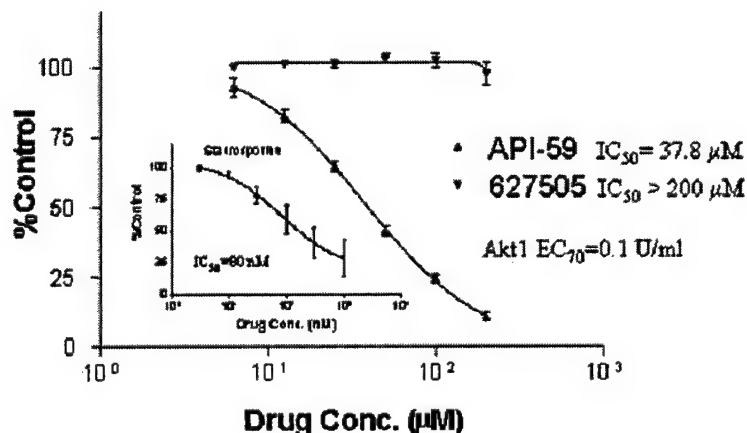


Fig. 5 Fluorescence polarization-based IMAP TM AKT1 assay. Different Concentrations of API-59 or NSC627505 were subjected to IMAP TM kinase assay against AKT1.

The IMAP Akt Assay kit (Molecular Device, Sunnyvale, CA) is designed to evaluate kinase activity of Akt. It uses IMAP technology for non-antibody fluorescence polarization (FP) detection of phosphorylation. The IMAP technology is based on the high affinity binding of phosphate by immobilized metal (MIII) coordination complexes on nanoparticles. This IMAP "binding reagent" complexes with phosphate groups on phosphopeptides generated in a kinase reaction. Such binding causes a change in the rate of the molecular motion of the peptide, and results in an increase in the fluorescence polarization values observed for the fluorescein label attached at the end of the peptide. Specifically, first to get the EC₇₀ value of Akt by preparing an enzyme dilution curve with adding 5 μ l of Complete Reaction Buffer, serial diluted Akt enzyme, fluorescein-labeled substrate (final concentration 0.1 μ M), and ATP (final concentration 5 μ M) in one well of a 384-well plate (Corning, NY), reaction at room temperature for 1 hr, adding 60 μ l IMAP binding reagent, reaction at room temperature for 30 min, then reading the plate with Tecan Ultra microplate reader set at fluorescence polarization mode. Second to get IC₅₀ values of candidate Akt inhibitors by setting up the same assay with serial dilution of inhibitors against Akt with the activity of EC₇₀. Purified Akt1 enzyme is now commercially available (Upstate). With this method, we directly detect the inhibitory effect of API-59 in regard to its inhibition against Akt1-mediated SGK substrate phosphorylation. As seen in **figure 5**, positive control staurosporine inhibits Akt1-mediated SGK substrate phosphorylation with IC₅₀ 80 nM; while API-59 shows an inhibitory effect against Akt1 with IC₅₀ 37.8 μ M. Compound NSC627505 does not inhibit Akt1. This indicates that API-59 inhibits Akt1

Taken together, we identified a AKT inhibitor API-59, API-59 inhibits the activity of phospho-Ser473 AKT in a dose and time-dependent fashion. API-59 inhibits at least the activity of both AKT1.

2.2. API-59 Selectively Inhibits the Activity of AKT

To demonstrate that the lead compound selectively inhibit the AKT kinase but not other kinases, we used the same cell lysates from the above-mentioned experiments (HS578T treated with API-59) to probe with antibodies against phosphor-tyrosin kinases, phospho-specific MAP kinase, and PI-3 kinase. As shown in **Fig. 6**, API-59 does not inhibit

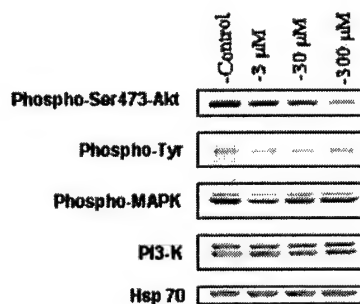


Fig. 6 API-59 inhibits only AKT, not inhibit protein tyrosine kinases, PI3K, or phospho-p42/p44 MAP kinase. 2×10^5 HS578T cells were treated with desired concentrations of API-59 for 30 min, cell lysates were harvested and subjected to AKT kinase assay and Western blot analysis. Hsp70 reprobing is showing equal loading of the total protein samples.

these target proteins. These results suggest that **API-59** inhibits AKT activity in cancer cells with high-levels of AKT activity, API-59 selectively inhibits only the AKT kinase activity, does not affect the upstream protein kinases of AKT or MAP kinase.

2.3. Increased Concentration of ATP Reverses the AKT inhibitory effect of API-59

AKT/PKB is a serine/threonine kinase, ATP is required for its function. To clarify whether ATP interferes with the AKT inhibitory effect of API-59, we performed an AKT kinase assay with different concentrations of GSK-3 fusion protein and ATP in the presence of various concentrations of API-59, using the AKT immunoprecipitated from HS578T cells. As demonstrated in **figure 7**, at 1 μ g of GSK-3 fusion protein: without API-59, ATP obviously increased AKT kinase activity in a dose-dependent manner, shown by phosphor-GSK-3 band; with 0.4 and 2.0 μ g/ml of API-59, ATP still increased AKT kinase activity; with 30 μ M API-59, ATP did not increase AKT kinase activity. At 0.5 μ g of GSK-3 fusion protein: without API-59, ATP still obviously increased AKT kinase activity in a dose-dependent manner; with 0.4, 2.0, and 10 μ g/ml of API-59, ATP increased AKT kinase activity. This finding indicates the interference of ATP to the AKT inhibitory effect of API-59 is decided by the concentration of API-59 and ATP, and the concentration of the substrate GSK-3 protein for the kinase reaction, ATP and the AKT inhibitory effect of API-59 interfere with each other under certain condition. This result suggests that API-59 might also be a kinase inhibitor.

2.4. API-59 Inhibits Ligand-induced Activation of AKT

As a cell survival factor, AKT can be activated by a variety of growth factors and cytokines.

To investigate whether API-59 works under this condition, we tested the ability of API-59 in blocking

heregulin (HRG) induced AKT activation in MCF-7 cells as described [63]. As shown in **figure 8A**, treatment with Heregulin 30 nM for 30 min readily induced activation of AKT in MCF-7 cells, which usually expresses low or undetectable level of AKT, treatment of these cells again with 30 μ g/ml of API-59 significantly reduced such activation. Furthermore, as predicted, blocking of AKT activation also resulted in subsequent reduction of phosphorylation of pro-apoptotic protein BAD, which is down-stream substrate of AKT (**figure 8B**). If this effect in cells by API-59 is due to its ability to block the AKT activation, this effect would not affect proteins in a different pathway. To test this hypothesis, we then tested the ability of API-59 in inducing the activation of MAP

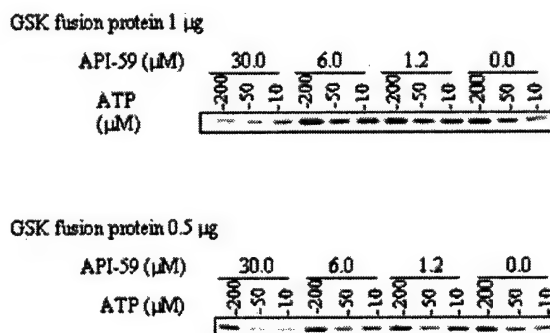


Fig. 7 The AKT inhibitory effect of API-59 can be reversed by ATP. 250 μ g of HS578T cell lysates were used to immunoprecipitated AKT, then subjected to kinase assay with desired concentrations of ATP and GSK-3 fusion protein in the presence of different concentrations of API-59.

kinase in the same system. As shown in figure 8B, heregulin readily induced activation of MAP kinase, which is consistent with reports in the literature and our previous data [64]. However, API-59 had no effect on the MAP kinase activation induced by ligand heregulin. These results illustrate that API-59 selectively inhibits ligand-induced activation of AKT.

2.5. API-59 Selectively Inhibits the Growth of Cancer Cells with High AKT Activity

AKT/PKB transmits

the survival/over-growth signal in cancer cells, to investigate whether API-59 can block this signaling process by inhibiting the growth of cancer cells with high AKT activity, we performed a serial growth assays to test the effect of API-59 on cell proliferation. Cells were treated with a series of doses of API-59 with regular culture medium supplemented with 10% FBS for 5-7 days and then cell viability was determined by Cell Counting Kit-8 [44]. The rationale of this kit is similar to that of the MTT assay, but with a novel

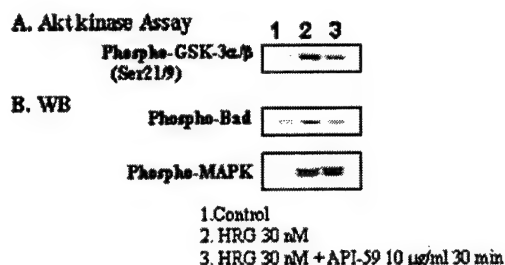


Fig. 8 API-59 inhibits heregulin-induced activation of Akt. **A:** Heregulin induces activation of AKT in MCF-7 cells, but this activation is inhibited by API-59. 3×10^5 MCF-7 Cells were treated as indicated in the picture, cell lysates were harvested and subjected to AKT kinase assay. **B:** API-59 inhibits heregulin-induced up-regulation of phospho-Bad, but does not affect the activation of phospho-MAP kinase. Same lysates were subjected to Western blot analysis with phospho-Bad and phospho-p42/p44 MAP kinase specific antibodies, respectively

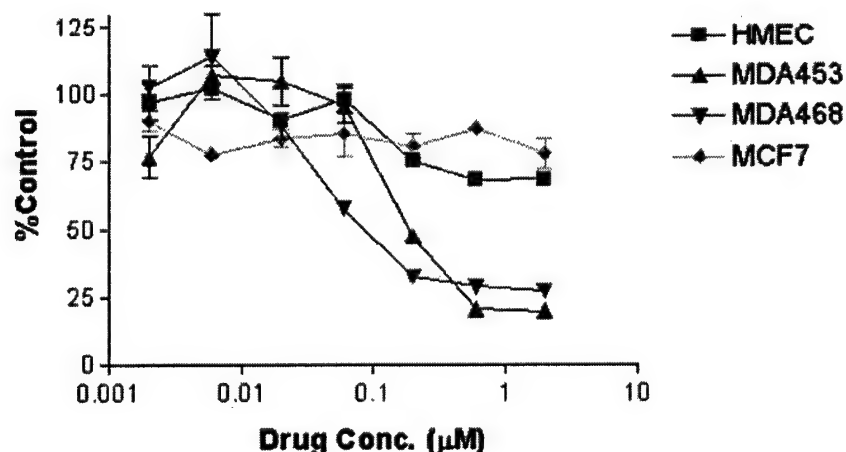


Fig. 9 API-59 inhibits the growth of cancer cells with high Akt activity. 5×10^5 MDA-MB-453, MDA-MB-468, and MCF-7 breast cancer cells were treated with different concentrations of API-59 for 5 days, then cell viability was tested with Cell-Counting Kit-8.

tetrazolium salt that produces a water-soluble formazan dye upon bioreduction. As seen in **figure 9**, MDA-468 was the most sensitive cell line to the growth inhibitory effect of API-59. MDA-453 was moderately sensitive, whereas MCF-7 was resistant to this inhibitory effect. Among these breast cell lines, MDA-468 has the highest level of AKT activity, MDA-453 has a moderately high level of Akt activity, MCF-7 has no or undetectable level of AKT activity. These findings demonstrate that API-59 selectively inhibits the growth of cancer cells with high AKT activity.

2. 6. API-59 Selectively Induces Apoptosis of Cancer Cells with High AKT Activity

Apoptosis, or programmed cell death, is a very important content of cell death. Cancer cells with high AKT activity are so sensitive to the growth inhibitory effect API-59, it would be of great significance to see whether API-59 induces apoptosis of cancer cells. With TUNEL staining, which specifically stains the breakage of DNA, we observed that treated with 2 μ M of API-59 for 72 hours, there was almost no cells undergoing apoptosis in NIH3T3 cells which has no AKT activity, as shown in **figure 10B**; but more than 55% MDA-MB-453 cells which show a very high level of AKT activity, were undergoing apoptosis, as observed under microscope, as almost all the nuclei in treated MDA-MB-453 cells was caused DNA fragmentation, as shown in **figure 10D**. In untreated cells, we did not observe this morphological change (**figure 10A, 10C**).

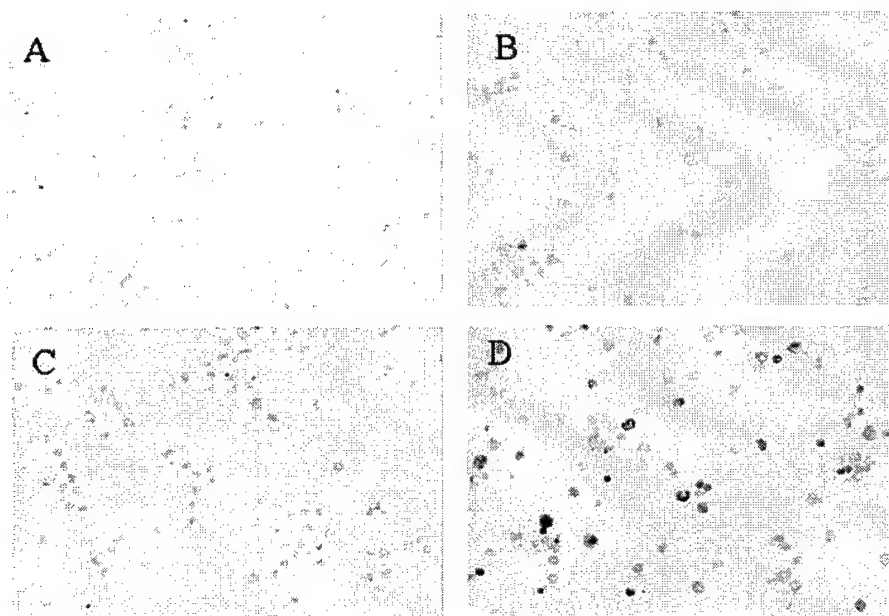


Fig. 10 API-59 induces apoptosis of cancer cells with high AKT activity. 1×10^6 MDA-MB-453 cells (high AKT activity) and 5×10^5 NIH3T3 cells were treated with 2 μ M API-59 for 72 hrs, cells were then subjected to TUNEL staining. **A**. Untreated NIH3T3 cells. **B**. Treated NIH3T3 cells. **C**. Untreated MDA-MB-453 cells. **D**. Treated MDA-MB-453 cells.

Conclusively, API-59 selectively induces apoptosis in cancer cells with high AKT activity, it is a potent apoptosis-inducing reagent.

Accomplishments on Objective 3: *in vivo* antitumor activity and toxicity

We have synthesized enough of API-59 for *in vivo* antitumor activity and toxicity study.

REPORTABLE OUTCOMES:

1. Meeting Abstract

FASEB Summer Research Conference- Protein Kinases and Protein Phosphorylation, July 19-24. Snomass, CO

Specific blockade of AKT/PKB activity in cancer cells with a selective AKT/PKB inhibitor API-59 (Appendix 1)

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2. Manuscripts:

- A. Discovery of a selective AKT/PKB inhibitor 9-chloro-2-methylellipticinium acetate (API-59) (Appendix 2)
- B. Selective Blockade of AKT Activity in Prostate Cancer Cells with 9-chloro-2-methylellipticinium acetate (API-59) (Appendix 3)

3. Colloborations:

We have provided data and materials for Dr. Jiayu Lin at the University of Michigan Cancer Center and helped to obtained two grants funding on effects of API-59 for endometrial cancer and ovarian cancer. There are two manuscripts coming out from this collaboration which we are co-authors.

4. There is one patent application is being drafted and is pending.

CONCLUSIONS

We have obtained an AKT-selective small molecule inhibitor, API-59 with both structure- and bioinformatics-approaches. API-59 selectively inhibits AKT, not the other upstream protein kinases of AKY or MAP kinases. API-59 inhibits the growth and induces apoptosis of cancer cells with high-levels of AKT activity, but shows no activity towards normal cells without AKT activity.

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